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Computer-controlled *in-vitro* simulation of multiple dosing regimens

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The bactericidal effect of gentamicin on *Pseudomonas aeruginosa* ATCC 27853 was investigated in a computer controlled dynamic *in-vitro* model, which allows the simultaneous simulation of three different dosing regimens for several days. The same total dose reduced cfu-counts of *Pseudomonas aeruginosa* most effectively, when administered with peak concentrations of 32 mg/l every 32 h, whereas the other dosing regimens with peak concentrations of 16 mg/l every 16 h and 8 mg/l every 8 h were distinctly less effective following the second and subsequent doses. It was shown that the use of a microcomputer facilitates the *in-vitro* investigation of multiple dosing regimens but counting of cfu cannot be substituted by automatic measurements of turbidity when rapid bactericidal effects occur.

Introduction

With few exceptions, such as gonorrhoea or uncomplicated urinary tract infections, the treatment of infectious diseases requires administration of antibiotics in multiple doses for several days. It therefore seems appropriate to perform *in-vitro* investigations of multiple dosing regimens which can yield important information concerning the bactericidal or bacteriostatic action of the second or subsequent doses and the possibility of emerging resistance, phenomena which escape the *in-vitro* studies of single doses.

The use of a microprocessor with the capacity to control simultaneously multiple independent sets facilitates the performance of long-term experiments and improves their reproducibility. The feedback of certain data to the computer offers additional advantages such as (i) information concerning the actual state of the experiment by displaying and recording turbidity data which can be used as a guideline for diluting the samples for cfu-determinations and (ii) maintenance of constant pharmacokinetic conditions by verification of liquid levels.

Turbidity data can further be used to initiate automatically active intervention in an experiment, e.g. when lack of action of a drug is detected

The purpose of the present study was to prove the feasibility of the computer controlled *in-vitro* model to determine the effect of three different multiple dosing regimens of gentamicin on the viable counts of *Pseudomonas aeruginosa*.

Materials and methods

Media

Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA) were obtained from Difco (Brunschwig AG, Basel, Switzerland). MHB was supplemented with Mg^{++} and Ca^{++} (MHB-S) according to NCCLS standards (1983).

Antibiotics

Gentamicin laboratory reference powder was obtained from Schering (Essex Chemie, Luzern, Switzerland).

Bacterial strain

Log-phase cultures of *Ps. aeruginosa* ATCC 27853 were photometrically adjusted by diluting them with MHB-S to yield a final inoculum of 10^5 cfu/ml. Glass flasks (volume 125 ml) were filled with 100 ml of the bacterial suspension. The minimal inhibitory concentration (MIC) of gentamicin in MHB-S was 2.0 mg/l.

Viable counts of bacteria

To detect > 100 cfu/ml, three drops of 20 μ l, each from appropriate tubes of serial ten-fold dilutions, were plated on MHA. Less than 100 cfu/ml were quantified by membrane filtration of 1 ml and 10 ml of undiluted samples, yielding a sensitivity of 0.5 cfu/ml. Filters were washed with 100 ml of 0.9% NaCl in water to eliminate residual drug and placed on MHA. Viable counts were not adjusted for the loss which occurred through the dilution with drug free broth.

Simulation of multiple dosing regimens

The *in-vitro* model (designated apparatus model I) described by Grasso *et al.* (1978) was modified by adding a second peristaltic pump which drained the culture flask and thereby maintained a constant fluid level (Figure 1, pump 3). In addition, this pump

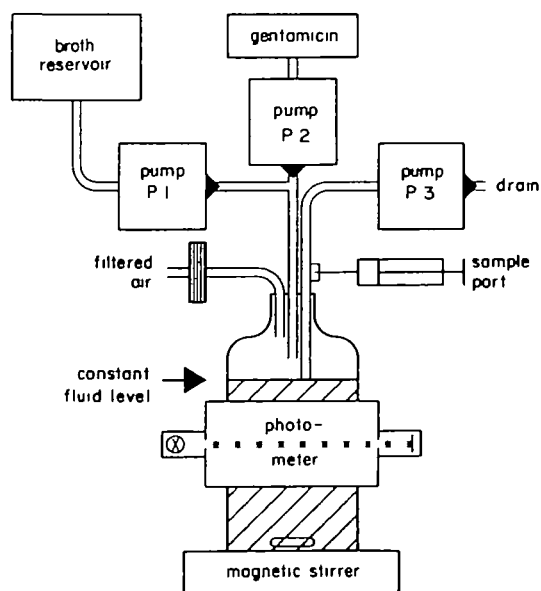


Figure 1. Single set of the *in-vitro* model. Pump speed of pump P1 was adjusted to produce the desired elimination half-life, whereas pump P3 (drain) was suctioning at a higher flow rate to maintain the constant volume and to provide the ventilation of the head space with filtered air (pore size: 0.2 μ m). Pump P2 was operated according to the dosage regimens.

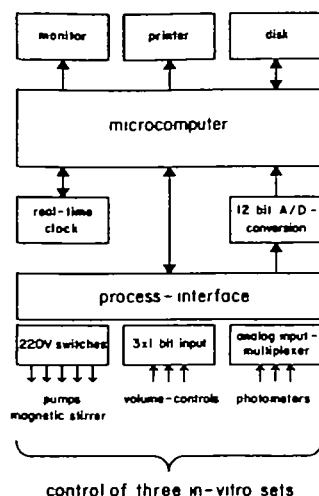


Figure 2. Diagram of the computer system. The process interface was designed to control simultaneously three *in-vitro* sets as shown in Figure 1.

provided continuous ventilation of the head space of the culture flask through a port for filtered air which allowed aerobic growth conditions. Three sets of the *in-vitro* model were simultaneously operated in an incubator at a temperature of 37.0°C, simulating three multiple dosing regimens (A, B and C) with identical total doses, which produced peak gentamicin concentrations of 8, 16 and 32 mg/l at dosage intervals of 8, 16 and 32 h, respectively. The infusion periods were 30 min each. Human one-compartment pharmacokinetics with an elimination half-life of 2.2 h were simulated by continuous dilution with drug free broth.

Turbidimetry

Three photometers were built with extra-bright light-emitting diodes, wave-length 660 nm (Stanley ESBR 5501, Dewald AG, Zürich, Switzerland), matched pairs of photodiodes (United Detector Technology PIN-3DP, Traco AG, Zürich, Switzerland) and a log ratio amplifier (LOG100, Burr-Brown AG, Rüschlikon, Switzerland) providing a compensated operation with a threshold for the detection of > 200,000 cfu/ml. Turbidity was measured in each culture flask over a path length of 4.6 cm and recorded on the disc of the computer at 5 min intervals.

Computer control

An Apple II® plus computer (Apple Computer Inc., Cupertino, California), supplied with a specially designed interface was used to control simultaneously three sets of the *in-vitro* model as shown in Figure 2.

Results

Multiple dosing regimens

The gentamicin concentration versus time curves for the three different dosing regimens are displayed in Figure 3. The resulting viable counts and optical density

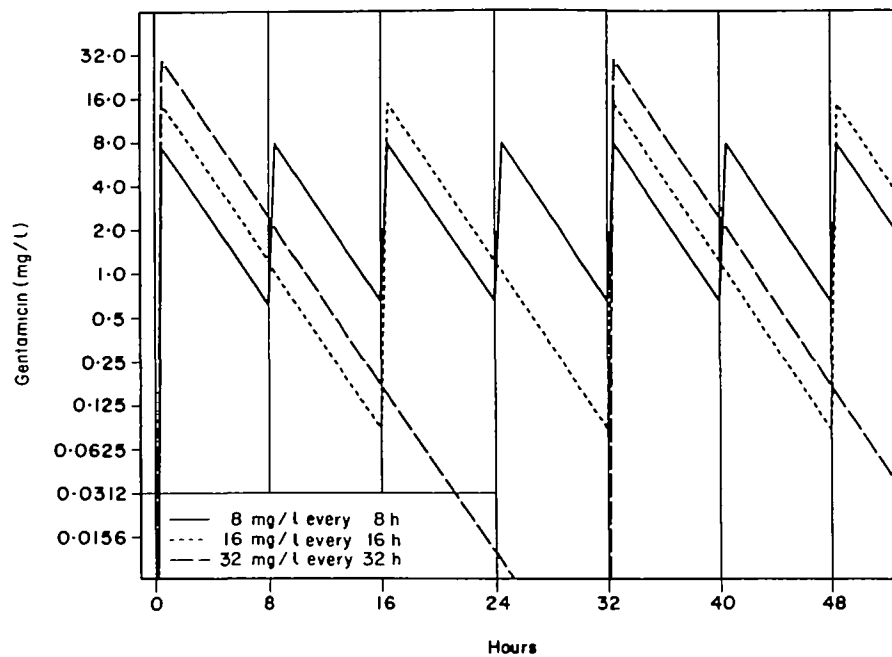


Figure 3. Concentration versus time curves of the three gentamicin dosing regimens A, B and C with an identical total dose yielding peak concentrations of 8, 16 and 32 mg/l at intervals of 8, 16 and 32 h, respectively

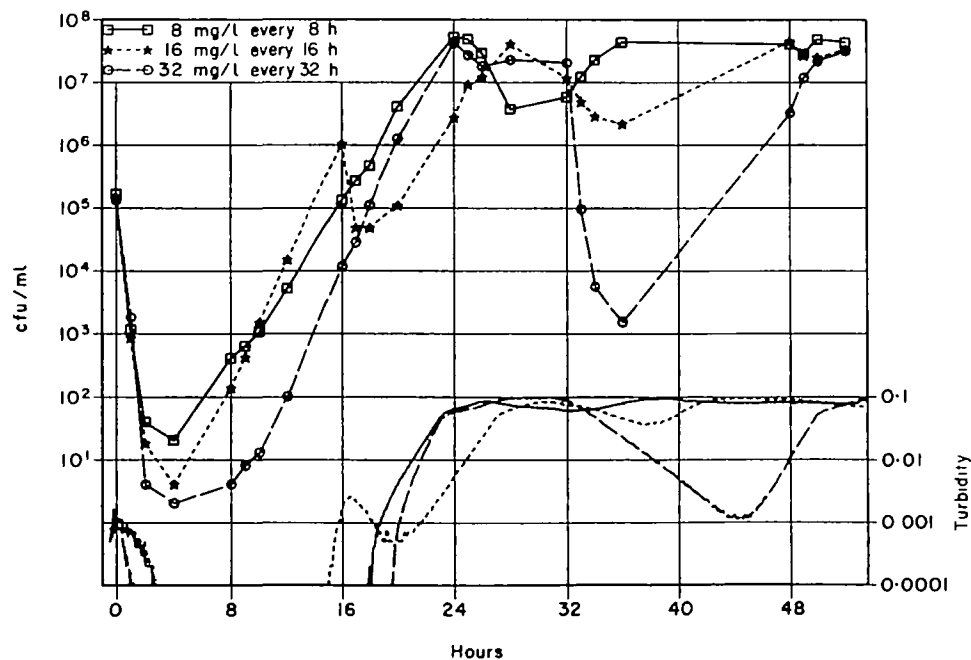


Figure 4. Cfu and turbidity versus time curves of *Ps aeruginosa* ATCC 27853, exposed to the three dosing regimens of gentamicin which are displayed in Figure 3. The photometers, operated at 660 nm, yielded a threshold of the detection of > 200,000 cfu/ml

versus time curves (Figure 4) show a rapid and dose dependent decline after the first dose, followed by regrowth within 4 to 8 h. Apart from a slight reduction of cfu at 24 h, the subsequent doses of regimen A (8 mg/l peak concentrations every 8 h) showed no apparent bactericidal activity. The second and third dose of regimen B, yielding a peak concentration of 16 mg/l at 16 and 32 h were marginally effective, and at 48 h, the effect of the fourth dose was completely lacking. This is in contrast to the obvious bactericidal effect of the second dose of regimen C (32 mg/l peak concentration) at 32 h which resulted in a decrease of approximately 4 log cfu, comparable to the effect of the first dose.

Turbidity versus time curves showed a good correlation with the cfu-counts when growth occurred. In periods of rapid bactericidal action (e.g. regimen C at 32 h), turbidity was determined by the washout of dead bacteria through dilution with drug free broth and deviated markedly from the determinations of viable counts.

Discussion

The effect of multiple doses of gentamicin on *Ps. aeruginosa* ATCC 27853 was studied in a computer controlled *in-vitro* model. It was demonstrated that identical total doses of gentamicin were most effective, when administered at intervals of 32 h. Although the concentration of gentamicin for that regimen (C) was below the MIC for approximately 3/4 of the dosage interval, cfu's did not exceed the counts observed with regimen A within the first 24 h. Indeed, the long period of subinhibitory concentrations may be the reason for the retained bactericidal action of the second dose.

The on-line measurement of turbidity showed a good correlation with > 200,000 cfu/ml as long as growth occurred and was very useful as a guideline for diluting the samples for cfu-determinations. Due to insufficient sensitivity and the inability to quantify rapid bactericidal effects, as observed with aminoglycosides, the substitution of cfu-counts by turbidity measurements is not advisable.

The use of a microcomputer was a time-saving and reliable method to simulate multiple dosing regimens for several days. Limitations in simultaneously performing multiple dosing regimens were found to be the processing of samples, increasing number of pumps and space in the incubator.

Further development of the model includes the analogue control of pump speeds to simulate combination therapies of drugs with different half-lives and the automatic use of turbidity data to evaluate optimal timing of synergistic combinations.

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